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Fluconazole Non-Susceptible Breakthrough Candidemia after Prolonged Low-Dose Prophylaxis: a Prospective FUNGINOS Study

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Running title : Breakthrough candidemia: a nationwide study of FUNGINOS.

Key words : candidemia, breakthrough, fluconazole, susceptibility, *Candida*, species, FUNGINOS.

Highlights:

- FUNGINOS conducted a nationwide prospective study of candidemia in Switzerland.
- Breakthrough candidemia (BTC) occurred in 8% of 567 consecutive candidemias.
- BTC was observed in hemato-oncological patients with gastrointestinal mucositis.
- Prolonged low-dose fluconazole prophylaxis was associated with non-susceptible BTC.
- Severity of infection and mortality were not increased in BTC compared to non-BTC.

Statistics

Title (100 characters) : 107
Running title (50 characters) : 49
Key words : 7
Highlights (3-5 bullet points, 85 characters incl. spaces / bullet point) : 5 (81, 78, 82, 85, 84)
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Tables : 2
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Abstract

Objectives. Breakthrough candidemia (BTC) on fluconazole was associated with non-susceptible *Candida* spp. and increased mortality. This nationwide FUNGINOS study analyzed clinical and mycological BTC characteristics.

Methods. 3-year prospective study in 567 consecutive candidemias. Species identification and susceptibility testing (CLSI) in reference laboratory. Data analysis according to STROBE criteria.

Results. 43/576 (8%) BTC were studied: 37/43 (86%) on fluconazole (28 prophylaxis, median 200mg/day). 21% BTC vs. 23% non-BTC presented severe sepsis/septic shock. Overall mortality was 34% vs. 32%. BTC was associated with gastrointestinal mucositis (multivariate OR 5.25, 95%CI 2.23-12.40, p<0.001) and graft-versus-host-disease (6.25, 1.00-38.87, p=0.05), immunosuppression (2.42, 1.03-5.68, p=0.043), parenteral nutrition (2.87, 1.44-5.71, p=0.003). Non-*albicans Candida* were isolated in 58% BTC vs. 35% non-BTC (p=0.005). 63% of 16 BTC occurring after 10-day fluconazole were non-susceptible (*Candida glabrata, Candida krusei, Candida norvegensis*) vs. 19% of 21 BTC (*C. glabrata*) following shorter exposure (7.10, 1.60-31.30, p=0.007). Median fluconazole MIC was 4mg/l vs. 0.25mg/l (p<0.001). Ten-day fluconazole exposure predicted non-susceptible BTC with 73% accuracy.

Conclusions. Outcome of BTC and non-BTC was similar. Fluconazole non-susceptible BTC occurred in three out of four cases after prolonged low-dose prophylaxis. This implies reassessment of prophylaxis duration and rapid de-escalation of empirical therapy in BTC after short fluconazole exposure.

Introduction

Candida spp. belong to the top ten bloodstream pathogens in the hospital [1, 2]. Candidemia is associated with 40-80% overall mortality, 5-70% attributable mortality, prolongation of hospital stay, and substantial hospital costs [3-6]. Azole prophylaxis in high-risk patients resulted in a decreasing incidence of candidemia in some hospitals [7]. A concomitant emergence of non-*albicans Candida* spp. with decreased susceptibility or intrinsic resistance to fluconazole, in particular *Candida krusei* and *Candida glabrata,* has been described. This shift has been reported in breakthrough candidemia (BTC) occurring on fluconazole [8-17]. A microbiological study in *Candida* isolates from BTC described a correlation between dose of fluconazole and minimal inhibitory concentration (MIC), without reporting related clinical data [18]. In BTC, guidelines recommend the empirical change to an echinocandin or liposomal amphotericin B, regardless of the duration of fluconazole exposure [19-21]. There is a need for more information on clinical and mycological characteristics of BTC and their impact on patients' management and outcome.

The objective of this nationwide prospective study from the Fungal Infection Network of Switzerland (FUNGINOS) was to characterize the patients at risk of BTC, the relationship between antifungal exposure, *Candida* spp. and antifungal susceptibility as well as the clinical severity and outcome of BTC.

Methods

Patients.

Consecutive episodes of candidemia were prospectively studied over a three-year period (2004-2006) in university (n=5) and university-affiliated centers (n=20) of the FUNGINOS network covering hospital care for 85% of the Swiss population. First episodes of candidemia were analyzed; relapsing or recurrent episodes were excluded.

Patients with candidemia were prospectively identified by microbiology laboratory investigators. Clinical investigators collected data on patients' demographics, risk factors, management, and outcome in a paper print report form. Data were centralized to the national study coordination. After queries to investigators, the final clinical reports were validated by the FUNGINOS data review committee (members listed in the section "Authors' contributions").

BTC was defined as candidemia occurring during or up to 48 hours after stopping antifungal prophylaxis or treatment of a duration of at least three days [18].

The study has been approved by the Ethical Committee of the Lausanne University Hospital as the national coordinating center.

Candida bloodstream isolates.

The microbiology laboratories of the 25 centers used automated blood culture systems (Bactec™ [Becton Dickinson, Sparks, Maryland, USA] or BacT/Alert® [bioMérieux, Marcy l'Etoile, France]). The *Candida* bloodstream isolates were centralized to the FUNGINOS mycological reference laboratory (Institute of Microbiology, Lausanne University Hospital). *Candida* species were identified by standard biochemical assays in a test gallery (ATB ID 32 C®, bioMérieux, Marcy l'Etoile, France) [22]. In case of discordant species identification between center and reference laboratory, molecular identification was performed by PCR amplification and sequencing of the D1/D2 region of the large subunit of the 28S ribosomal RNA gene (28S rDNA). Antifungal susceptibility testing was performed by microtitre broth dilution method with the Sensititre® YeastOne™ test panel (TREK Diagnostic System, Cleveland, Ohio, USA). Minimal inhibitory concentrations (MIC) were interpreted according to

clinical breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) [23]. Candida isolates with MIC fullfilling CLSI criteria for dose-dependent susceptibility and resistance were considered as non-susceptible.

Data analysis

The analysis was designed according to the guidelines from the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [24].

Data were analyzed using the SPSS 17.0 statistical software package (SPSS Inc., Chicago, IL, U.S.A.). Categorical data were compared by Chi-square or Fisher's exact test, and continuous variables by T-test or Mann-Whitney test. Uni- and multivariate logistic regression analyses were performed to assess the association of clinical variables with the occurrence of BTC. All variables with a p-value <0.2 in the univariate analysis were included in a multivariate logistic regression model using a step-wise forward likelihood ratio procedure, which implies that only variables significantly associated with BTC remained in the final multivariate model. Collinearity of variables in the final model was estimated using the variance inflation factor: a value <2 was considered as reflecting the absence of significant collinearity. Point estimates were reported as odds ratios (OR) with 95% confidence intervals (95%CI). A two-sided p-value <0.05 was considered statistically significant.

Results

567 consecutive episodes of candidemia were reported. Forty-three episodes (8%) fullfilled the pre-defined criteria for breakthrough candidemia (BTC), while 524 (92%) did not (non-BTC). 550 (97%) *Candida* blood isolates were sent to the FUNGINOS reference mycology laboratory.

Among 43 BTC episodes, 37 (86%) occurred on fluconazole and 6 (14%) on another antifungal (3 voriconazole, 2 caspofungin, 1 itraconazole).

Patients' demographics and clinical characteristics in BTC and non-BTC are summarized in **Table 1**. Multivariate analysis identified independent associations of gastrointestinal (GI) tract mucositis, acute gastrointestinal GvHD, immunosuppressive drugs, and total parenteral nutrition with BTC.

The proportions of *Candida* spp. in BTC vs. non-BTC are summarized in **Table 2**.

After detection of BTC, 46% of patients were treated with fluconazole and 54% with another antifungal drug, while in non-BTC, 85% of patients received fluconazole and 15% an alternative regimen (p<0.001).

Severe sepsis or septic shock was reported in 21% of BTC episodes vs. 23% of non-BTC (p=0.85). 34% of patients with BTC died vs. 32% with non-BTC (p=0.73).

Thirty-seven BTC episodes occurred on fluconazole (76% prophylaxis, 24% therapy). The median duration of fluconazole exposure was 11.5 days (3-62) in prophylaxis vs. 5 days (4-17) in therapy (p=0.008). The median daily dose of fluconazole prophylaxis was 200 mg (50-800) and the median cumulative dose 2'900 mg (600-21'600).

14/37 (38%) *Candida* spp. were non-susceptible to fluconazole (dose-dependent susceptible or resistant): 13/14 (93%) were isolated during fluconazole prophylaxis. The rate of fluconazole non-susceptibility increased with the duration of fluconazole exposure (**Figure 1, Panel A**). A fluconazole expoure during 10 days or longer occurred in 43% of BTC and predicted a fluconazole non-susceptible *Candida* isolate with 71% sensitivity, 74% specificity, 63% positive predictive value (PPV), 82% negative predictive value (NPV), and 73%

accuracy (ROC curve **Figure 1, Panel B**). BTC occurring on fluconazole during more than 10 days was more often caused by non-*albicans Candida* spp. when compared with a shorter exposure (13/16, 81% vs. 6/21, 29%; OR 10.90, 95%CI 2.25-52.20, p=0.001) **(Figure 2).** Among 16 BTC episodes after prolonged fluconazole exposure, 10 (63%; 5 *C. glabrata*, 4 *C. krusei*, 1 *C. norvegensis*) were non-susceptible, whereas among 21 after shorter exposure, 4 (19%; 4 *C. glabrata*) were non-susceptible (OR 7.10, 95%CI 1.60-31.30; p=0.007)**.** The median MIC of fluconazole in BTC isolates was 4 mg/l (range 0.125-128) when exposure exceeded 10 days vs. 0.25 mg/l (range 0.125-32) after shorter exposure (p<0.001).

7/23 patients with fluconazole susceptible BTC died (overall mortality 30%) vs. 5/14 patients with fluconazole non-susceptible BTC (36%, p=1.00), respectively. Fluconazole was continued during more than three days in 9/14 (64%) BTC episodes due to non-susceptible isolates and switched to an appropriate antifungal regimen within three days in 5/14 (36%): overall mortality was 56% (5/9) and 0% (0/5), respectively (p=0.085).

Discussion

This FUNGINOS study was designed on a large prospective sample of candidemias representing the nationwide epidemiology in Switzerland. Its unique characteristic was the integration of validated individual clinical and drug dosing data with mycological data from the national FUNGINOS reference laboratory. BTC due to non-susceptible non-*albicans Candida* spp. occurred in three out of four cases in hematological patients with toxic damage of the GI-tract receiving prolonged low-dose fluconazole prophylaxis. This observation from a robust clinical and mycological dataset provides a proof of principle of what has been described in previous reports focused on either clinical or mycological data. Implications of this finding for antifungal stewardship are: i) regular reassessment of the indication and duration of fluconazole prophylaxis for reducing the emergence of difficult to treat breakthrough infections requiring expensive parenteral regimens, ii) once species and antifungal susceptibility is known, de-escalation from empirical therapy for BTC in a majority of patients with short fluconazole pre-exposure.

FUNGINOS observed BTC in 8% of 567 consecutive candidemias which occurred in an unselected hospital patient population from 25 hospitals. A retrospective survey of 409 candidemias from 6 hospitals reported 9% of BTC [15]. The CANDIPOP investigators reported 15% of BTC among 237 candidemias in cancer patients [17]. A retrospective study in surgical patients with intra-abdominal infections reported 15% of BTC [16]. Other authors observed higher BTC proportions, up to 72% in hemato-oncological patients [10, 15, 25]. Different case-mix and prophylaxis policies explain this large variability in the incidence of BTC.

In the FUNGINOS study 76% of BTC occurred on fluconazole prophylaxis, while rates in other surveys ranged 57%-100% [15][16][17]. GI-tract mucositis following cytotoxic chemotherapy, acute gastrointestinal GvHD, immunosuppression, and total parenteral nutrition were found to be independently associated with BTC [15, 17, 26-29]. Candidemia occurs in hemato-oncological patients after disruption of the intestinal mucosal barrier and invasion by *Candida* spp. colonizing the GI-tract [30, 31]. Fluconazole prophylaxis is

recommended in this high-risk setting, which explains the association of these conditions with BTC [32].

Our study offers a proof of principle by reporting the clinical-microbiological relationship between prolonged low-dose fluconazole prophylaxis in high-risk hemato-oncological patients with toxic damage of the GI-tract and occurrence of BTC due to non-susceptible non-*albicans Candida* spp.. The selective pressure exerted on the endogenous flora highlights the importance of a regular reassessment of the duration of fluconazole prophylaxis for reducing the emergence of difficult to treat BTC. Other studies reported 40%-48% of non-susceptible *Candida* spp. in BTC, without providing dosing information [15][17]. *C. krusei* and *C. glabrata* candidemia was observed in hemato-oncological patients on fluconazole prophylaxis, whose duration was not described [33-35]. A mycological report by Pfaller *et al.* reported an association of fluconazole exposure with non-susceptible candidemia [36]. High fluconazole MICs were observed in non-*albicans Candida* spp. from BTC [25, 37][38]. Other reports did not associate fluconazole prophylaxis with a shift in the *Candida* species: different epidemiological and clinical settings might explain these contrasting results [39-41]. A report by Clancy et al. from U.S. hospitals correlated fluconazole doses (>200 mg/d, >2000 mg cumulative) with dose-depent susceptible or resistant BTC, without related clinical data [18].

Rates of severe sepsis/septic shock (around 20%) and overall mortality (around 30%) did not differ in BTC and non-BTC. Data from the literature range 10%-80% and 30-60%, respectively [15-17, 25, 27]. Fluconazole susceptibility did not impact on mortality in BTC [32, 37]. While empirical antifungals other than fluconazole were more frequently prescribed in BTC, a trend to higher mortality was observed in patients with fluconazole non-susceptible BTC who were not promptly switched to an alternative regimen. Other authors recorded a reduced survival in patients with fluconazole-resistant BTC [4, 17, 38]. Despite variability in case-mix and clinical practices, these observations suggest that a strict application of guidelines recommending empirical switch to an alternative antifungal therapy improves outcome in BTC.The small number of BTC episodes is a limitation which might have resulted

in underpowered statistical analyses. Recent multicenter epidemiological surveys from Europe, South and North-America, analyzed similar samples sizes of BTC: the consistency of our findings with previous reports corroborates their robustness [15, 17, 29, 32, 37, 38]. Although the validity of data collected in 2004-2006 might be debated, FUNGINOS showed that *Candida* species distribution and antifungal susceptibility remained stable over a 20-year period ([1, 42], unpublished data). Based on this epidemiology, our analysis was designed in a unique, carefully validated, clinical and mycological dataset prospectively collected nationwide over a three-year period. In Switzerland, the increase of the use of echinocandins during the following decade was moderate, and fluconazole remains the first-line agent for antifungal prophylaxis and by far the most frequently prescribed antifungal (unpublished data). Alltogether these points support that the study findings are representative of the current epidemiology of BTC in Switzerland.

In conclusion, severity and outcome of BTC and non-BTC were not different. Prolonged low-dose fluconazole prophylaxis in hemato-oncological patients with toxic damage of the GI-tract was associated with BTC due to azole-non susceptible non-*albicans Candida* spp.. This finding implies a regular reassessement of the duration of prophylaxis. Once species and MICs are known, a de-escalation from empirical antifungal therapy for BTC is possible in the majority of patients with short fluconazole pre-exposure.

Transparency declarations

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All other co-authors : no financial disclosure.

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Authors' contributions

O.M. designed, implemented, and coordinated the candidemia cohort study.
J.G., S.Z., A.I., K.B., U.F., C.O., A.C., T.Bre., and O.M. collected clinical data, together with the clinical investigators from the centers of the FUNGINOS network listed in the Appendix.

J.S., K.M., R.Z., T.Bru., R.F., and J.B. collected *Candida* blood isolates and performed species identification and antifungal susceptibility testing, together with the clinical microbiologists from the centers of the FUNGINOS network listed in the Appendix.
J.B. and F.L. coordinated the FUNGINOS reference mycology laboratory.
O.M. coordinated the Data Review Committee composed of K.B., T.Bre., U.F., J.G., A.I., S.Z.
C.O., D.M., J.B. and O.M. organized the dataset and performed statistical analyses.
C.O., D.M., J.B. and O.M. wrote the manuscript, with the help of S.Z., L.D., N.K., A.C., V.E., P.Y.B., T.C., F.L., S.E., C.VD., C.R., and J.F.
All authors critically revised the manuscript and accepted the final version submitted for publication.

Appendix.

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Figure 1.

Panel A. Duration of Fluconazole Exposure at the Time of Breakthrough Candidemia and Rate of Non-Susceptible *Candida* isolates.

Thirty-seven episodes of breakthrough candidemia occurred during fluconazole prophylaxis or therapy.

Panel B. Duration of Fluconazole Exposure at the Time of Breakthrough Candidemia as Predictor of Non-Susceptible *Candida* Isolates.

Figure 2.

Proportions of Isolated *Candida* spp. According to the Duration of Fluconazole Exposure at the Time of Breakthrough Candidemia (BTC).

Non-*albicans Candida* spp. were more frequently isolated after fluconazole exposure exceeding 10 days (p=0.001).

Table 1.

Uni- and Multivariate Analysis of Demographic and Clinical Characteristics Associated with Breakthrough Candidemia.

Table 2.

Candida species distribution comparing breakthrough (BTC) and non-breakthrough candidemia (Non-BTC).